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(54) **Cell smear staining composition and material, and production and use thereof**

(57) The composition comprises haematein, a triphenylmethane dyestuff (preferably light green) and a fluorescein dyestuff (preferably eosin). The composition is preferably in the form of a dry, homogeneous (preferably solution-applied) film coated on a support, so as to constitute a cell smear staining material.

The composition and material are used for staining cell smears (for cytodiagnosis) in a single staining step by mordanting the smears with a metal salt solution and then contacting the smears with the composition. This staining method is simple and (when the cell staining material is used) clean.

GB 2 026 015 A

SPECIFICATION

Cell smear staining composition and material, and production and use thereof

The invention relates to cell smear staining. For decades (more than 30 years) the standard method of staining cell smears in gynaecological cytodiagnosis for the early recognition of genital cancer in women has been the Papanicolaou stain. The principle of this method is that vaginal, portio and cervical smears, and also uterine sediment smears, which contain individual cells or cell groupings, are stained with several dyestuff solutions. On the basis of cell morphology, cancer cells or precursors thereof can be recognised.

The disadvantages of this staining method are that it is very time-consuming and many years' experience are required in order to be able to evaluate the results obtained. The Papanicolaou stain method involves 23–25 steps, including covering of the preparation; the time taken is about 30–45 minutes. The actual staining procedure involves more than 10 steps (3 staining solutions and rinsing with water, alcohol, acid and base). Attempts have been made to simplify the time-consuming staining operation and to shorten the time taken so that gynaecologists could use the method in their own practices without having to send smears to an outside, specialist cytological laboratory.

A large number of modifications and rapid staining techniques have been proposed for the Papanicolaou stain, but have only achieved limited acceptance. Papanicolaou was aware of the disadvantages of his method and he described in *J. Lab. Clin. Med., 1941,* page 1,200, a staining solution consisting of aniline blue, orange G, fuchsin, eosin, molybdatophosphoric acid and tungstophosphoric acid, which is said to provide a stain usable for many cases in only 3 minutes; however, without the use of haematoxylin, the nuclei are not stained so well and cytological details are not so easily discernible. Papanicolaou gave a similar report on his solutions EA 36 and EA 25, which consist of light green, Bismarck brown, eosin, tungstophosphoric acid and lithium carbonate (*Science, 95,* 2469 (1942)). In *ann. Biol. Clin., 12,* 187 (1954), the Papanicolaou stain is compared with seven other methods, showing that although single stains stain the cytoplasm very well, they are not able to show the structure of the nucleus. In every case, at least an additional stain with haematoxylin is necessary.

We have now developed a staining composition with which it is possible, in a single staining step, to obtain images which correspond to the Papanicolaou stains, both in respect of the fine structure and in respect of the colour character.

According to the invention, there is provided a composition for staining cell smears, which comprises haematein, a triphenylmethane dyestuff (preferably light green) and a fluorescein dyestuff (preferably eosin).

The composition may be in the form of a solution which preferably contains 0.01–0.5% by weight of haematein, 0.01–0.5% by weight of the triphenylmethane duestuff and 0.05–1% by weight of the fluorescein dyestuff. Such a solution may be used directly for staining cell smears, but since solutions generally do not have unlimited stability and their composition can change if they are used repeatedly, for example, as a result of partial evaporation of the solvent or due to crystallisation of a constituent, it is advantageous to apply these solutions to a support and to evaporate the solvent to form a dry homogeneous film of the composition according to the invention. Suitable supports for such coatings are, for example, glass, plastics films or absorbent supports, such as paper or glass fibre paper.

The resulting coated material is particularly useful for staining cell smears, since the staining can be carried out cleanly and simply without the need to store dyestuff solutions with its associated difficulties.

The composition according to the invention is preferably used in a process of staining smear samples present on a sample-receiving surface (such as, for example, a microscope slide), as follows.

The smear samples should be fixed immediately in conventional manner, for example, by spraying with a commercially available fixative or by dipping in an ethanol/ether mixture.

In order that the cell nucleus should be stained, the fixed smear should be mordanted. For this purpose, the smear sample is treated with a metal salt solution, for about 3 minutes, for example. Suitable metal salts include, for example, iron salts, bismuth salts, copper salts and aluminium salts, the latter being preferred. Preferably, a complexing agent, such as citric acid, tartaric acid, gluconic acid, ethylenediaminetetraacetic acid or the corresponding salts, is added to the metal salt solution so as to reduce or avoid the formation of precipitates. The solution preferably contains the metal salt in a concentration of about 0.1–5% by weight. With lower concentrations, the nucleus is stained hardly any more deeply than the plasma and with high salt concentrations coloured precipitates are likely to be formed in the plasma.

The treated smear samples are then contacted with a composition according to the invention, which may be in the form of a solution or dry (for example, when present as a coating on a support). When the composition is applied dry, the samples should still be moist following

treatment with the metal salt solution. A suitable time for contact with the composition according to the invention is about 5 minutes.

When the composition according to the invention is used in the form of a coating on a substrate, the substrate can be removed subsequently, if desired. The stained smear is then preferably rinsed with alcohol and covered.

With the preferred composition according to the invention (that is a composition comprising haematein, light green, and eosin in the preferred proportions indicated above), the colour pattern of the resulting stains corresponds closely to that of Papanicolaou stains. Slight colour shifts can arise if the ratios of dyestuffs to one another are changed or if, for example, phloxin or erythrosin is used in place of the preferred fluorescein dyestuff, eosin, or if, for example, patent blue is used in plalce of the preferred triphenylmethane dyestuff, light green.

In order that the invention may be more fully understood, the following Examples are given by way of illustration only:–

Example A

Preparation of the solutions

1. Metal salt solution

0.5 g of aluminium chloride and 0.5 g of sodium citrate in 100 ml of water

2. Staining composition

The following 3 solutions are prepared and filtered:

(a) 1.20 g of haematein in 300 ml of ethanol

(b) 0.69 g of light green in 20 ml of water and 100 ml of ethanol

(c) 2.88 g of eosin in 30 ml of water and 100 ml of ethanol.

30 ml of solution (a), 12 ml of solution (b) and 13 ml of solution (c) are combined to give a staining composition.

Example B

Preparation of a cell smear staining material

The staining composition prepared according to Example A2 is applied uniformly to a 55 mm wide and 1,000 mm long film of glass-clear polyvinyl chloride (thickness 0.15 mm), for example by applying with a sponge or by spraying or printing. After the solvent has evaporated, the film is cut into strips 24 mm wide and can then be used for staining.

Example C

Staining procedure

A vaginal, cervical or uterine smear on a microscope slide is fixed immediately by placing the slide in a 1:1 mixture of ethanol/ether for about 10–15 minutes. The smear can also be fixed by spraying with a fixative spray. The smear fixed in this way is dipped in the metal salt solution described in Example A1 for about 3 minutes. The staining material prepared according to Example B is placed on the smear, whilst the latter is still moist. After 5 minutes the film is removed and the smear is rinsed with alcohol and covered in conventional manner.

The cell constituents of the smear are observed in the microscopic image to be stained as follows:

Basophilic cytoplasm	blue-green
Acidophilic cytoplasm	pink
Erythrocytes	red
Cornified plasma	pink to orange
Cell nuclei	blue to dark violet

CLAIMS

1. A composition for staining cell smears, which comprises haematin, a triphenylmethane dyestuff and a fluorescein dyestuff.

2. A composition according to claim 1, in which the triphenylmethane duestuff is light green.

3. A composition according to claim 1 to 2, in which the fluorescien dyestuff is eosin.

4. A composition according to any of claims 1 to 3, which is in the form of a solution containing 0.01–0.5% by weight of haematein, 0.01–0.5% by weight of the triphenylmethane dyestuff and 0.05–1% by weight of the fluorescein dyestuff.

5. A composition for staining cell smears, substantially as herein described in Example A.

6. A cell smear staining material, which comprises a support having thereon a dry homogeneous film of a composition according to any of claims 1 to 3.

7. A cell smear staining material, substantially as herein described in Example B.

8. A method of producing a cell smear staining material, which comprises applying a solution of a composition according to any of claims 1 to 3 to a support and removing the

solvent to form a dry homogeneous film of said composition on the support.

9. A method according to claim 8, in which the solution is as defined in claim 4.

10. A cell smear staining material, when produced by a method according to claim 8 or 9.

11. A process of staining fixed cell smear samples present on a sample-receiving surface, which comprises mordanting the samples with a metal salt solution and then contacting the samples, while they are still moist, with a composition according to any one of claims 1 to 3 or with the dry homogeneous film of a material according to claim 6, 7 or 10.

12. A process of staining fixed cell smear samples present on sample-receiving surfaces, which comprises treating the samples with a metal salt solution and contacting the samples with a composition according to claim 4 or 5.

13. A process of staining cell smears, substantially as herein described in Example C.

14. For use in cytodiagnosis, a composition according to any of claims 1 to 5 or a material according to claim 6, 7 or 10.

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